



PATENT  
0030-0200P

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Applicant: Marcus KEEP et al. Conf.: 1549  
Appl. No.: 09/674,092 Group: 1653  
Filed: February 27, 2001 Examiner: MOHAMED, A.A.  
For: CEREBROSPINAL AND VASCULAR  
PHARMACEUTICAL COMPOSITION AND PROCESS  
FOR PREPARING THE SAME

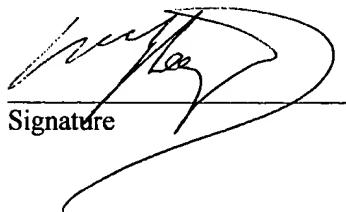
DECLARATION UNDER 37 C.F.R. § 1.132

I, Marcus Keep, M.D., declare and state

- 1) I am a graduate of the Medical University of South Carolina, Charleston, SC where I received my medical degree in the year 1988. My residency training in Neurosurgery was completed in 1994 at the Montreal Neurological Institute, McGill University, Montreal, Canada. I am Board Certified in Neurosurgery in both the United States and Canada. I had post-residency training as a research fellow at Lund University, Lund, Sweden from 1994 to 1996.
- 2) Since 1996, I have been in practice for 8 years as a neurosurgeon. I am Assistant Professor in the Division of Neurosurgery at the University of New Mexico Health Sciences Center, School of Medicine, where I teach neurosurgery residents and medical students in addition to providing neurosurgical care to patients.
- 3) I am familiar with the Office Action dated December 3, 2003.
- 4) I am one of the inventors of the above-identified application.

- 5) I am also familiar with Lebel et al. (Int. Arch. Allergy Immunol., Vol. 116, pp. 284-287, (1998), Kessler et al. (Biochemical Pharmacology, Vol. 40, pp. 169-173, (1990), and Falk '834 (US Patent No. 5,827,834) that have been cited by the Examiner in the Office Action of December 3, 2003.
- 6) In my opinion as a physician, no one has ever or would be given a drug dissolved in a deuterated dimethylsulphoxide (i.e., cyclosporin in deutero-DMSO) as a pharmaceutical preparation as disclosed in Kessler et al.
- 7) It is also my opinion as a physician that it would be very difficult if not impossible to administer a fluid volume of over 29 liters (7.6 gallons) a day to a person as would be required if one administered the concentration of cyclosporin in DMSO as disclosed in Lebel et al.
- 8) It is my opinion that Falk '834 uses DMSO to reduce brain swelling and hyaluronic acid as a carrier and to enhance penetration in the compositions disclosed in Falk '834 (see column 10 lines 25-30 in Falk '834).
- 9) It is my opinion that the instant invention would be unexpected in light of the disclosures of Lebel et al., Kessler et al., and Falk '834.

I hereby declare all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under 18 USC §1001, and that such willful false statements may jeopardize the validity of the application or any patent that issues therefrom.



Signature

29 Feb 04  
Date

## Solubilization of Cyclosporin A

Submitted: November 20, 2000; Accepted: January 15, 2001

Yingqing Ran,<sup>1</sup> Luwei Zhao,<sup>2</sup> Qing Xu,<sup>3</sup> Samuel H. Yalkowsky<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, College of Pharmacy, The University of Arizona, Tucson, AZ 85721

<sup>2</sup>Aventis Pharmaceuticals Company, Bridgewater, NJ 08807

<sup>3</sup>Department of Chemistry, The University of Arizona, Tucson, AZ 85721

AAPS PharmSciTech 2001; 2 (1) article 2

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**ABSTRACT** This study investigated the solubilization of cyclosporin A (CsA), a neutral undecapeptide, by cosolvency, micellization, and complexation. Cosolvents (ethanol, propylene glycol, polyethylene glycol, tetrahydrofurfuryl alcohol polyethyleneglycol ether, and glycerin), surfactants (polyoxyethylene sorbitan monooleate [(Tween 80)], polyoxyethylene sorbitan monolaurate [(Tween 20)], and Cremophor EL), and cyclodextrins ( $\alpha$ -cyclodextrin [ $(\alpha$ CD)] and hydroxypropyl- $\beta$ -cyclodextrin [(HP $\beta$ CD)]) were used as solubilizing agents in this study.

Surfactants had a noticeable effect in increasing CsA solubility. Twenty percent solutions of Tween 20, Tween 80, and Cremophor EL increased the solubility by 60 to 160 fold. Cyclodextrins can increase the CsA solubility, but  $\alpha$ CD was more effective than HP $\beta$ CD. Cosolvents on the other hand did not increase the solubility of CsA as much as expected from the LOGP (logarithm of water-octanol partition coefficient) value of CsA.

**KeyWords:** Cyclosporin A, Conformation, Solubility, Solubilization

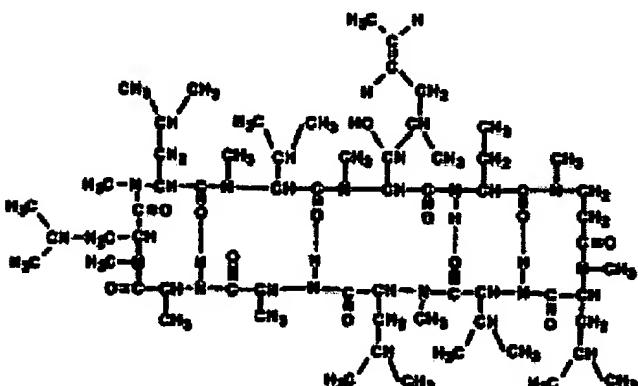


Figure 1. Structure of cyclosporin A as established by Rügger and Petcher [1].

\*Corresponding Author: Yingqing Ran, PhD; Department of Pharmaceutical Sciences; College of Pharmacy, The University of Arizona; 1703 Mabel Street; Tucson, AZ 85721; Telephone: 520-626-3847; Facsimile: 520-626-4063; E-mail: [ran@pharmacy.arizona.edu](mailto:ran@pharmacy.arizona.edu)

## INTRODUCTION

Cyclosporin A (CsA; see Figure 1) [1] is an effective immunosuppressive agent used in transplantation to prevent organ rejection. The aqueous solubility of CsA is 27.67  $\mu$ g/mL at 25°C [2, 3]. This study investigated the use of the traditional solubilization approaches to increase the solubility of CsA.

The solubility of the drug is determined by the interaction of solute with solvents and the crystallinity of the solute. Solvent alteration is the most effective means to produce a thermodynamically stable increase in solubility [2]. As discussed by Yalkowsky [4], the 3 most commonly used approaches for solubilizing nonionizable drugs such as CsA are cosolvency, micellization, and complexation.

### Cosolvency

Cosolvents are organic compounds that are substantially miscible with water. Cosolvents have small hydrocarbon regions. Since these regions are nonpolar and they do not interact strongly with water, they can reduce the ability of the aqueous system to squeeze out nonpolar solutes. The logarithmic relationship between total drug solubility ( $S_{\text{mix}}$ ) in a mixed solvent and cosolvent concentration ( $C_{\text{cosol}}$ ) can be described by equation 1 [4-6].

$$\log S_{\text{tot}}^{\text{mix}} = \log S_w + \sigma C_{\text{cosol}} \quad (1)$$

where  $S_w$  is drug solubility in water and  $\sigma$  is cosolvent solubilization power. The  $\sigma$  value depends on the polarity of both the solvent and the solute. The more nonpolar the solvent and the solute, the larger the  $\sigma$  value.

### Micellization

Organic solutes can be solubilized by incorporation into the micelles formed by surfactants. The more nonpolar the solute, the more likely it is to be incorporated near the core or center of the micelle. The

relationship between the drug solubility in a micellar solution and surfactant concentration is described by equation 2a [4, 7, 8].

$$S_{\text{tot}}^{\text{mic}} = S_w + \kappa(C_{\text{surf}} - \text{CMC}) \quad (2a)$$

where  $C_{\text{surf}}$  is the concentration of micellar surfactant (ie, the total surfactant concentration minus the critical micellar concentration) and  $\kappa$  is the molar solubilization capacity, the number of moles of solute that can be solubilized by 1 mole of micellar surfactant. If the critical micellar concentration (CMC) is much lower than  $C_{\text{surf}}$ , equation 2a can be approximated by

$$S_{\text{tot}}^{\text{mic}} \approx S_w + \kappa C_{\text{surf}} \quad (2b)$$

### Complexation

Cyclodextrins are cyclic oligosaccharides that have been recently used as complexation ligands. Cyclodextrins can interact with appropriately sized molecules to result in the formation of inclusion complexes. The effects of various cyclodextrins can be approximately described by equations 3 and 4 [4, 7, 9, 10].

$$\begin{aligned} S_{\text{tot}} &\approx S_w + K_{1:1} S_w L \\ S_{\text{tot}} &\approx S_w + K_{1:1} S_w L + K_{1:1} K_{1:2} S_w L^2 \end{aligned} \quad (3)$$

where  $L$  is the total ligand concentration. Equation 3 is applicable to 1:1 (drug:ligand) complexation, and equation 4 is applicable to 1:1 and 1:2 (drug:ligand) complexation.  $K_{1:1}$  is the complexation constant for 1:1 drug-ligand complex;  $K_{1:2}$  is the complexation constant for 1:2 drug-ligand complex. Higher-order complexes are also possible.

Since cyclosporin A does not have ionizable groups, we did not consider pH control to increase its solubility. But the other 3 approaches have the potential to be used to solubilize CsA.

## MATERIALS AND METHODS

### Materials

Cyclosporin A was a gift from the Institute of Microbiology, Fujian, China.  $\alpha$ -Cyclodextrin ( $\alpha$ CD) and hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD) were obtained from Cyclodextrin Technologies Development Inc (Gainesville, FL). All other chemicals were of analytical or high-performance liquid chromatography (HPLC) grade, purchased from Sigma and Aldrich (St. Louis, MO).

### Solubility Measurement

The CsA powder was added to vials containing various percentages of cosolvents, surfactants, or complexation agents. Duplicate sample vials were prepared for each solubilizing agent at its particular concentration and were placed on an end-over-end mechanical rotator at 25 rpm at room temperature for 7 days. Samples with drug crystals present were considered to have reached the equilibrium and were removed from the rotator. The samples were then centrifuged on the Micro16 centrifuge (Fisher Scientific, Pittsburgh, PA) at 12 000 rpm for 30 minutes. Supernatant was diluted before injection into the HPLC system.

The cosolvents used were ethanol (EtOH), propylene glycol (PG) and polyethylene glycol (PEG400), tetrahydrofurfuryl alcohol polyethyleneglycol ether (glycofurool), and glycerin. The surfactants were polyoxyethylene sorbitan monooleate (Tween 80), polyoxyethylene sorbitan monolaurate (Tween 20), and Cremophor EL. The complexation ligands were  $\alpha$ CD and HP $\beta$ CD. The concentration ranges are given in Table 1.

### HPLC Assay

A Beckman Gold (Fullerton, CA) system equipped with a model No. 168 detector at 215 nm was used for HPLC assay in this study. A pinnacle octylamine column 150 cm  $\times$  4.6 mm, (Restek, Bellefonte, PA) was used with a mobile phase composed of 70% acetonitrile and 30% water [11]. The flow rate is 1 mL/min. The temperature was elevated to 70°C by immersing the column in a waterbath. The retention time of CsA was 5.6 minutes. The injection volume was 50  $\mu$ L. The CsA standard curve at concentrations ranging from 5  $\mu$ g/mL to 100  $\mu$ g/mL was used to evaluate all of the assays.

## RESULTS AND DISCUSSION

The solubility values of CsA as functions of the cosolvent, surfactant, and cyclodextrin concentrations are presented in Figures 2, 3, and 4, respectively. The solubilization parameters described can be determined by fitting the data in these figures to equations 1 to 4. The resulting parameters are presented in Table 1, which gives the range of concentration of the solubilization agents used in this study and the solubilization parameters for CsA.

### Cosolvency

Figure 2 shows the exponential increase in CsA solubility with concentration of the cosolvent. The

value of  $\sigma$  here depends only on the polarity of the cosolvents. Table 1 shows that the less polar the cosolvent, the more effective the increase in the solubility of CsA and the larger value of  $\sigma$ . EtOH has the largest  $\sigma$  value and glycerin has the smallest. Although cosolvents can increase the solubility of CsA, their solubilization power is much lower than expected from the MLOGP value of CsA (MLOGP = 2.92) [12].

### Micellization

Figure 3 shows the effect of some surfactants on CsA solubility. Cremophor EL has the largest  $\kappa$  value. Tween 80 and Tween 20 produce similar effects on CsA solubility; if less than 10% is used, the solubility enhancement of CsA is relatively small. The slightly higher  $\kappa$  value of Tween 80 may be the result of its longer alkyl chains.

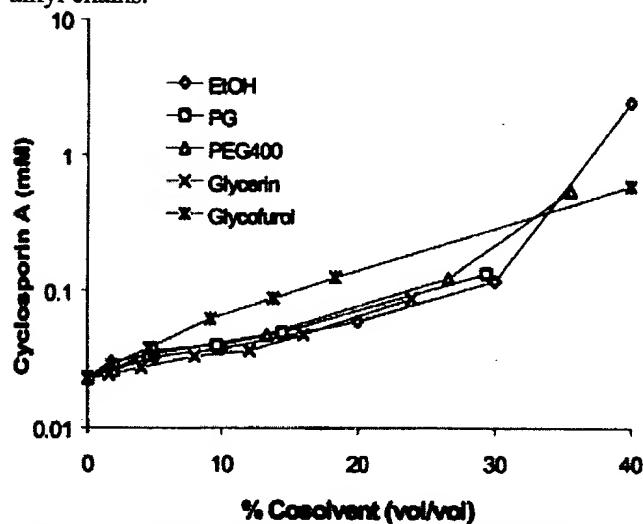


Figure 2. Effects of cosolvents on cyclosporin A solubility.

Table 1. Solubilization Parameters for Cyclosporin A

Solubilization Agents	Concentration Range, %	Parameters
EtOH	0-40 (vol/vol)	$\sigma = 0.044 \text{ %}^{-1}$
PG	0-40 (wt/vol)	$\sigma = 0.025 \text{ %}^{-1}$
PEG400	0-40 (wt/vol)	$\sigma = 0.035 \text{ %}^{-1}$
Glycofural	0-40 (wt/vol)	$\sigma = 0.031 \text{ %}^{-1}$
Glycerin	0-30 (wt/vol)	$\sigma = 0.023 \text{ %}^{-1}$
Tween 20	0-20 (wt/vol)	$\kappa = 0.0079$
Tween 80	0-20 (wt/vol)	$\kappa = 0.0119$
Cremophor EL	0-20 (wt/vol)	$\kappa = 0.0427$
$\alpha$ CD	0-20 (wt/vol)	$K_{1:1} = 47.8 \text{ M}^{-1}$ , $K_{1:2} = 18.22 \text{ M}^{-1}$
HP $\beta$ CD	0-20 (wt/vol)	$K_{1:1} = 21.7 \text{ M}^{-1}$ , $K_{1:2} = 6 \text{ M}^{-1}$

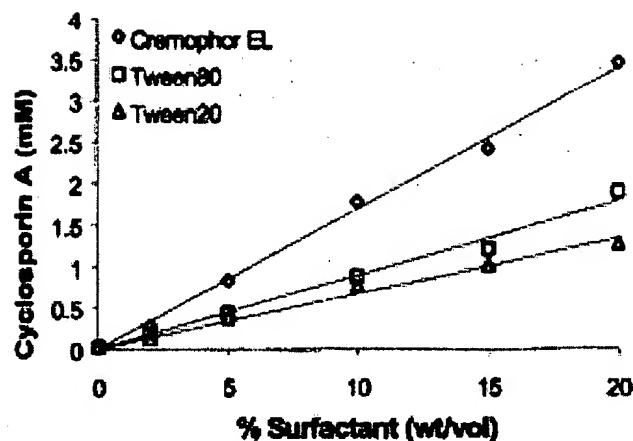


Figure 3. Effects of surfactants on cyclosporin A solubility.

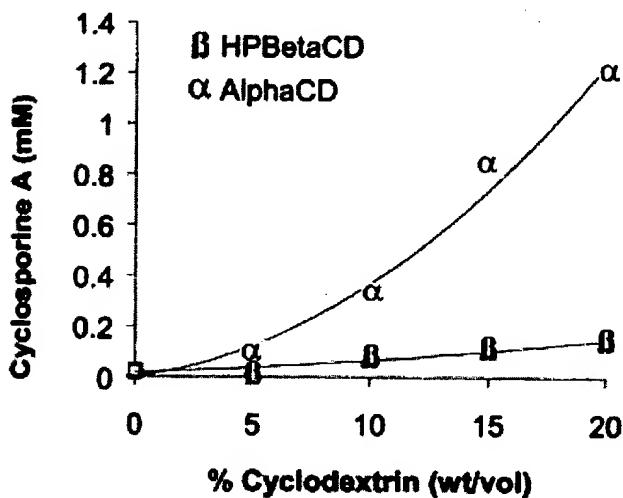


Figure 4. Effects of cyclodextrins on cyclosporine A solubility.

### Complexation

Figure 4 shows the effects of  $\alpha$ CD and HP $\beta$ CD on cyclosporine solubility. The CsA solubility does not show a linear rise as the function of the ligand concentration. It did not fit equation 3 but fit equation 4, suggesting that it forms both 1:1 and 1:2 complexes with both of the cyclodextrins. The bigger  $K$  value of  $\alpha$ CD is possibly a result of the smaller cavity (5 $\text{\AA}$ ), which is the more appropriate size for the nonpolar aliphatic parts of CsA to be held.

### CONCLUSIONS

This study investigated and compared 3 different approaches to solubilize CsA: cosolvents, surfactants, and cyclodextrins. The effectiveness of these approaches on CsA is not comparable to that of these approaches on most other nonpolar drugs. This may be the result of a conformational change in CsA structures

with solvent polarity (ie, the hydrogen bonds in Figure 1 would be favored by nonpolar media but they are not likely to exist in strongly hydrogen bonding media such as water). The NMR spectrum of CsA13 in CDCl<sub>3</sub> shows 4 doublets from 7 ppm to 8 ppm. This corresponds to the 4 intramolecular H-bonds that stabilize the conformation of CsA molecules in nonpolar media. The solubility of CsA in water is not sufficient to allow the study of the conformation of CsA in water, but the NMR spectrum of CsA in CD<sub>3</sub>OD does not contain the 4 doublets with high chemical shifts indicating the absence of intramolecular H-bonds of CsA in CD<sub>3</sub>OD. El Tayar et al [12] provided evidence of a conformational change of CsA in polar and nonpolar media from the structural information derived from both partitioning and simulation studies.

The structure in Figure 5 schematically illustrates the type of CsA conformation that may exist in water, whereas the structure depicted in Figure 1 illustrates its conformation in a nonpolar media. In water, the nonpolar parts of CsA are likely to associate with the polar parts pointed to the water molecules. This structure may make CsA behave somewhat like an unimolecular micelle, which has a higher affinity for water than would be expected from its structural composition.

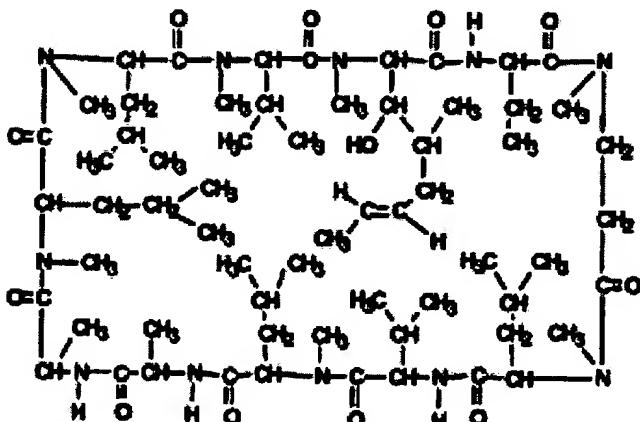


Figure 5. Schematic structure of cyclosporin A in water.

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